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European Journal of Advances in Engineering and Technology, 2018, 5(8): 604-608

Research Article ISSN: 2394 - 658X

The Effects of MIG1/2 and NRG1/2 on Trehalose Accumulation in Yeast *Saccharomyces cerevisiae*

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ABSTRACT

Trehalose is deposited by Saccharomyces cerevisiae as a storage carbohydrate and as a stress protectant. The regulation of trehalose level in yeast cell is strictly controlled by trehalose synthesis and degrading enzymes. The biosynthesis of trehalose is catalyzed by TPS complex and the breakdown of trehalose is catalyzed by neutral trehalase enzyme. The trehalose content of yeast cells increases in response to nutrient starvation and different environmental stresses. Mig1 and Mig2 are zinc-finger DNA binding transcription factors that are involved in glucose repression. Nrg1 and Nrg2 repressor proteins that bind to STRE and PDS elements on promoters, have also zinc-finger DNA binding domain in order to bind STRE and PDS elements on the promoters. Both Mig1/2 and Nrg1/2 are involved in regulation of genes controlled by glucose. In our research, the effect of Mig1/2 and Nrg1/2 repressor proteins on the accumulation of trehalose were investigated by using Δmig1, Δmig2, Δnrg1, Δnrg2 mutants and their isogenic wild-type yeast strain. The trehalose content of exponentially growing Δmig1 yeast cells was six fold higher than that of wild type and other mutant yeast cells. Nitrogen starvation triggered trehalose accumulation both in wild type and mutant yeast cells except Δmig1 mutant cells. Also the trehalose content of Δmig2, Δnrg1 and Δnrg2 mutant yeast cells were nearly four times higher than wild type in nitrogen deprivation. These results showed that Mig1 transcription factor is essential for maintenance of trehalose level both in standard and stress conditions, while Mig2, Nrg1 and Nrg2 repressor proteins are essential under stress conditions.

Key words: Mig1/2, Nrg1/2, Trehalose, *Saccharomyces cerevisiae*

INTRODUCTION

Trehalose, storage carbohydrate together with glycogen in *Saccharomyces cerevisiae*, is a nonreducing disaccharide. Trehalose is composed of two α-1,1 linked glucose monomers and this chemical bonding form enhances its stability. Highly stable structure makes trehalose useful for industrial and medical uses such as preservation and stabilization of biological storages. As found in yeast *S. cerevisiae*, trehalose is also found in many of other organisms from bacteria to fungi and plants [1, 2]. *TPS1* encodes trehalose-6-phosphate synthase, the first enzyme of trehalose biosynthetic pathway, and expression of *TPS1* is induced by the stress response. *NTH1* encodes trehalase enzyme that degrades trehalose into two glucose monomers. The regulation of *NTH1* expression is induced by various stresses as in *TPS1* gene regulation. Due to strictly regulated balance between biosynthesis and degradation of trehalose, intracellular trehalose plays an important role in the regulation of glycolysis [3]. *S. cerevisiae*accumulates trehalose under unfavourable environmental conditions. Accumulated trehalose is converted into glucose at the end of stress conditions and used as an energy source by the cells. Conversion of trehalose into glucose or vice versa is an important part of regulation of glycolytic pathway in yeast *S. cerevisiae* [4].

Nrg1 and Nrg2, zinc finger proteins and paralogs, are negative regulators of glucose-repressed genes including *SUC2*, *GAL*, *STA2*, *DOG2*, *FLO11* and mediate glucose repression in *S. cerevisiae* [5]. In addition to the regulation of glucose repressed genes, Nrg1 and Nrg2 both regulate several processes including response to oxidative and osmotic stress, alkaline pH, biofilm formation. Nrg1p also represses transcription by recruiting the Cyc8p-Tup1p complex to promoters [6, 7, 8]. The main effector of *SUC, MAL* and *GAL* genes in glucose repression is Mig1protein. When glucose is present, Zinc finger transcriptional repressor Mig1p represses transcription of genes that are responsible for utilizing alternative

carbon sources such as maltose, sucrose and galactose. Mig2p collaborates with Mig1p in repression of these genes. Transcriptional co-repressor Cyc8p-Tup1p is also recruited via Mig1p to promoters [9].
In this study, the effects of *MIG1/2* and *NRG1/2* genes on trehalose accumulation were analysed in *S. cerevisiae* both in normal and nitrogen starvation conditions. We showed that *MIG1* gene is essential both in normal and nitrogen starvation conditions. *MIG2*, *NRG1* and *NRG2* gene products are necessary for adjusting the trehalose level during starvation.

MATERIALS AND METHODS

Yeast strains and growth condition

The *MIG1* deletion strain Y04403 (*Mat a, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*. *YGL035c::kanMX4*), *MIG2* deletion strain Y04575 (*Mat a, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*. *YGL209w::kanMX4*), *NRG1* deletion strain Y03979 (*Mat a, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*. *YDR043c::kanMX4*), *NRG2* deletion strain Y03203 (*Mat a, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*. *YBR066c::kanMX4*) and its isogenic wild type strain BY4741 (*Mat a, his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*) were used in this study. All yeast strains were purchased from EUROSCARF (European *Saccharomyces cerevisiae* Archive for Functional Analysis).
In order to determine doubling times and specific growth rates of yeast strains, all yeast strains were grown in SD medium supplemented with auxotrophic requirements for 24 hrs at 30 °C with constant shaking (120 rpm). The optical densities of yeast cultures (OD) were measured spectrophotometrically by taking 1 ml sample from growing culture every two hours. The optical densities were used for calculation of doubling times (dt) and specific growth rates (μ) of yeast strains.

Application of starvation

All yeast strains were grown in SD medium supplemented with suitable amino acids, till prelogarithmic stage (OD600: 0.5-0.7) with constant shaking and temperature (30°C). Then yeast cultures were divided into two aliquots, and first aliquot was harvested and used for enzyme assay. Second aliquot was harvested, washed and transferred to fresh SD medium supplemented with auxotrophic requirements and 0.1% proline, instead of ammonium sulphate, and incubated at 30 °C. The cultures were incubated four hours more and harvested prior to determine trehalose contents.

Trehalose assay

Trehalose contents of yeast cells were determined as described previously [10]. Harvested yeast cells were washed with ice-cold water and then resuspended in 250 µl of 0.25 M Na_2CO_3 and boiled for 2 hours. Then 150 µl of 1 M acetic acid and 600 µl of 0.2 M sodium acetate pH 5.2 were added. Then cell mixture was incubated at 37 °C for 18 hr in the presence of 3mU trehalase enzyme (Sigma, T8778, 0.25U ml^{-1}) for trehalose assay. The amount of the liberated glucose was determined enzymatically via the glucose oxidase-peroxidase system (GOD-POD assay) using a commercial kit (Fluitest®- GLU, Biocon, Germany) [11]. The determined trehalose contents of yeast cells were given as microgram of glucose equivalent per milligram of wet mass (µg/mg) of the yeast cells. Results are given as mean values of three independent experiments that measured triplicate.

RESULTS AND DISCUSSION

Deletion of *MIG1* has more deleterious effect on trehalose accumulation

In order to determine the effect of Mig1 and Mig2 transcription factors on trehalose accumulation, the trehalose content of yeast cells was enzymatically hydrolysed into glucose and then released glucose was measured. The amount of trehalose was given as microgram glucose measured in mg cell wet mass (µg glucose/mg cell wet mass) of yeast cells. The amount of trehalose measured in *Δmig1*yeast cells (1615.5±55.7 µg glucose/mg cell wet mass) was 6-fold higher than wild type yeast cells (277.4±57.5 µg glucose/mg cell wet mass). However, the amount of trehalose measured in wild type yeast cells was twice as much as the amount of trehalose measured in *Δmig2* mutant yeast cells (148,72±30,6 µg glucose/mg cell wet mass) (Figure 1). Mig1 may downregulate TPS complex or upregulate *NTH1*, or both, for decreasing trehalose content in the yeast cell. These results indicate that Mig1, not Mig2, has an important role in regulating trehalose metabolism at normal nutrient abundant conditions.
The trehalose contents of *Δnrg1* and *Δnrg2* mutant yeast cells were determined as 92.2±78.3 µg glucose/mg cell wet mass and 253.2±68.1 µg glucose/mg cell wet mass, respectively (Figure 2). As shown that, the accumulation of trehalose in Δ*nrg1* yeast cells decreased nearly 3 fold than wild type, but there was no change in trehalose accumulation of Δ*nrg2* and wild type yeast cells under stress-free conditions. These results indicate that Nrg1 is essential for maintenance of basal trehalose level in the yeast cells

Nitrogen starvation triggers trehalose accumulation

Glutamine, glutamate, asparagine and ammonium are preferred nitrogen sources for *S. cerevisiae* yeast cells, while proline and urea are weak and poor nitrogen sources. Expression of genes involved in the uptake and utilization of poor nitrogen sources are suppressed in the presence of strong nitrogen source by the activity of nitrogen catabolite repression (NCR) mechanism [12, 13]. Tor signalling pathway which regulates *NTH1*, *TPS1* and *TPS2* gene expressions via Msn2/4 transcription factors, is regulated depending on the quantity and quality of nitrogen.
So we analysed the effects of poor nitrogen sources on trehalose accumulation in *Δmig1, Δmig2, Δnrg1, Δnrg2* and wild type yeast cells. First yeast cells were grown to logarithmic stage in the strong nitrogen source, ammonium, then washed

and transferred to poor nitrogen source, proline. After four hours' incubation, yeast cells were harvested and used for determination of trehalose content.

The accumulation of trehalose starts at diauxic shift and continues during stationary phase in S. *cerevisiae* yeast cells [14]. Also yeast cells accumulate trehalose in response to stress independent of growth stage. The trehalose content of wild type yeast cells (656.6±39.8 µg glucose/mg cell wet mass) increased 2 fold whenever nitrogen starvation was applied. The level of trehalose in *Δmig1, Δmig2, Δnrg1* and *Δnrg2* mutant yeast cells under nitrogen starvation was measured as 1153.3±28.5, 1759.2±127.3, 1515.6±164.1 and 2599.1±287.2 µg glucose/mg cell wet mass, respectively (Figure 3). It was determined that the amount of trehalose in mutant yeast cells increased two to four times greater than that of wild type. In all yeast strains nitrogen starvation triggers trehalose accumulation in different extends with regard to type of mutation.

The accumulation of trehalose in *Δnrg2* mutant yeast cells increased nearly 10 times more than in nitrogen rich condition. Similarly, in *Δnrg1* and *Δmig2* mutant yeast cells trehalose accumulation was nearly 17 and 12 times greater than that of non-starved cells, respectively. As opposed to high accumulation of trehalose in *Δnrg1, Δnrg2* and *Δmig2* mutant yeast cells, trehalose level in *Δmig1* mutant yeast cells did not changed during starvation.

CONCLUSION

Trehalose synthesis is accomplished by TPS (Trehalose Phosphate Synthase) complex enzyme. Stress-dependent trehalose degradation is carried out by Nth1. Neutral trehalase activity is dependent on the expression level of *NTH1* gene and the activation of trehalase by cAMP dependent PKA. The level of trehalose in yeast cells is regulated by the action of TPS complex and neutral trehalase enzyme depending on the signals coming from different pathways. The effects of Mig1 and Mig2 transcription factors and Nrg1 and Nrg2 repressor proteins on trehalose accumulation were summarized in Figure 4. Results of this study indicates that, Mig1 regulates the activity of both TPS complex and trehalase enzyme for decreasing the trehalose level. When *MIG1* gene is deleted, trehalose level increases both in the presence of good and poor nitrogen sources. It appears that *MIG2*, *NRG1* and *NRG2* gene functions are essential in the presence of poor nitrogen sources to maintain the trehalose level in the cell.

When we analyzed the promoters of NTH1 and TPS1 genes by using data bases and bioinformatics tools, we showed that both *TPS1* and *NTH1* promotor have at least three consensus binding sites for Nrg1 repressor protein and one consensus binding site for Mig1 and Mig2 transcription factors (data not given). These bioinformatics analyses also strongly support the role of Mig1 and Nrg1 proteins in the regulation of trehalose level. In order to identify the molecular mechanism of Mig1, Mig2, Nrg1 and Nrg2 effects on trehalose metabolism, further genetic and biochemical analysis must be conducted.

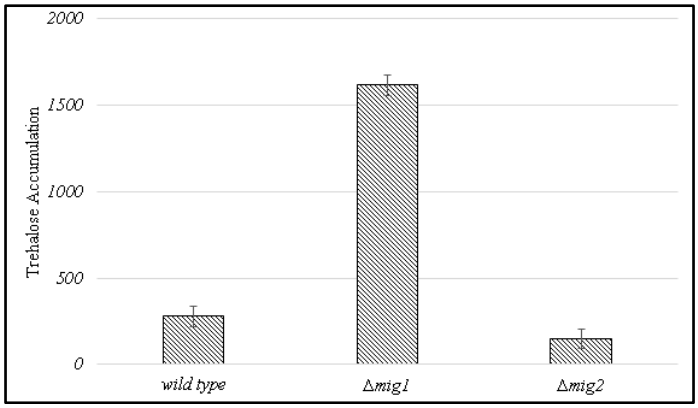

Fig. 1 Accumulation of trehalose in Δ*mig1* and Δ*mig2* mutants

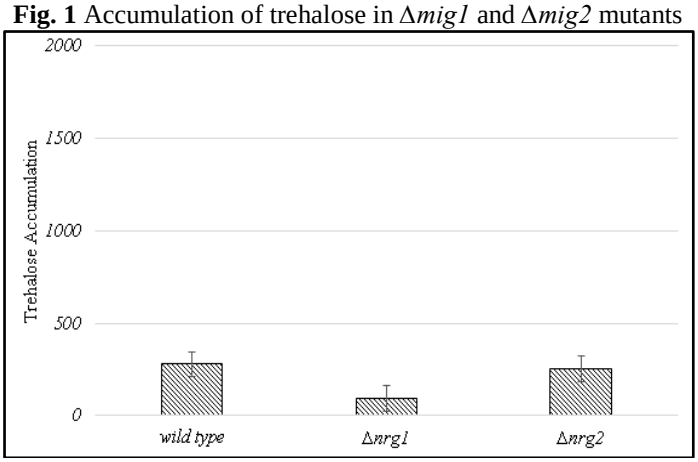

Fig. 2 Accumulation of trehalose in Δ*nrg1* and Δ*nrg2* mutants

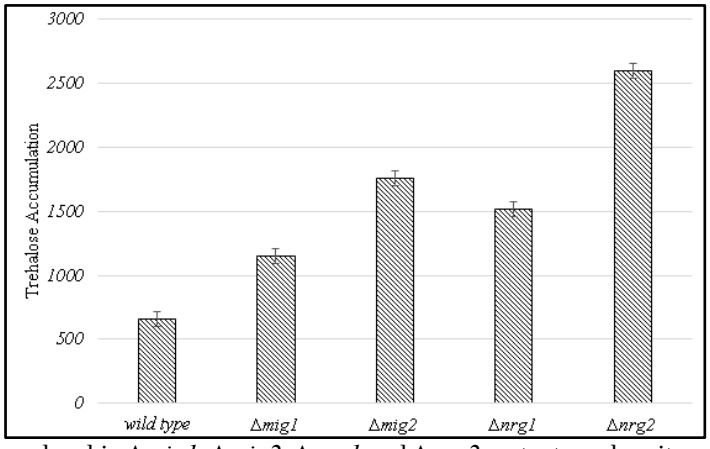

Fig. 3 Trehalose level in Δ*mig1*, Δ*mig2,* Δ*nrg1* and Δ*nrg2* mutants under nitrogen starvation

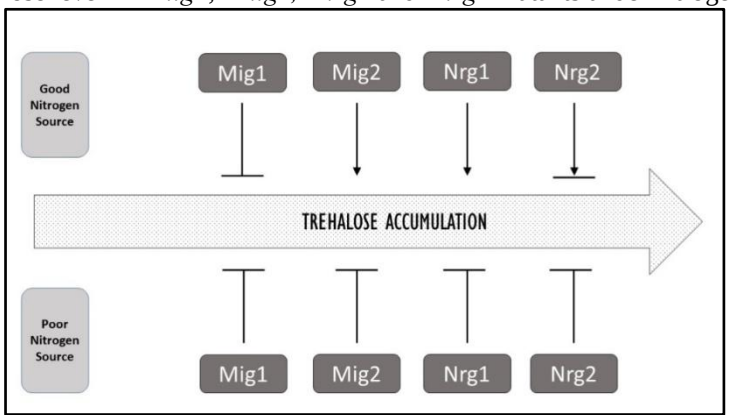

Fig. 4 The effects of Mig1, Mig2, Nrg1 and Nrg2 proteins on trehalose accumulation

Acknowledgements

This work was part of two different projects that supported by Çanakkale Onsekiz Mart University the Scientific Research Coordination Unit, Project numbers: FYL-2014-301 and FYL-2016-829. We thank to Prof. Dr. Sezai Türkel for his constructive and criticism of the manuscript.

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